Ciba Specialty Chemicals Corporation
USA

Textile Dyes

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8EHQ-1297-14090

Ciba

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December 15, 1997

EXPRESS MAIL

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RE: **TSCA Section 8(e) Notice; Sensitization and Mutagenicity Results with Disperse Orange 30**

Dear Section 8(e) Coordinator:

There is no confidential business information contained in this letter.

In accordance with EPA's March 16, 1978, policy statement on Section 8(e) reporting under the Toxic Substances Control Act and EPA's June 1991 TSCA Section 8(e) Reporting Guide, Ciba Specialty Chemicals Textile Dyes Division, wishes to bring to your attention sensitization results from a Guinea Pig Maximization Test, and mutagenicity results from a Salmonella typhimurium Reverse Mutation Assay. Chemically, Disperse Orange 30 is Propanenitrile, 3-{{2-(acetyloxy)ethyl}{4-{(2, 6-dichoro-4-nitrophenyl)azo}phenyl}amino}- (CAS No. 5261-31-4).

The allergenic potential of the test substance, Disperse Orange 30, was assessed in albino guinea pigs. Results of this study clearly indicate Disperse Orange 30 induced allergic skin responses in the Guinea pig: 10 of 10 animals showed grade 1 or 2 sensitization responses. The conclusion is clear that the test substance is a sensitizer under the study conditions.

The bacterial mutagenicity assay showed an increased rate of revertants in two of the test strains, but only one strain (TA98) showed revertant colony numbers well in excess of the positive control rate.

4050 Premier Drive
High Point, NC 27265

Tel. 910 801 2500

A copy of the final reports entitled "Contact Hypersensitivity to FAT 36141 in Albino Guinea Pigs Maximization-Test"(48 pp), and "Salmonella Typhimurium Reverse Mutation Assay with FAT 36141"(30 pp) is enclosed.

Please contact the undersigned if you require additional information.

Very truly yours,

Brett A. Rajkumar
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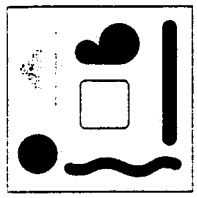
Encl.



RCC PROJECT 664694

CONTACT HYPERSENSITIVITY TO

FAT 36'141/C

IN ALBINO GUINEA PIGS
MAXIMIZATION-TEST

REPORT

Author:	G. Arcelin
Sponsor:	Ciba Specialty Chemicals Inc. TF 2.5 Dr. M. Studer K-424.2.06 Postfach CH-4002 Basel / Switzerland
Study Completion:	06-NOV-1997

RCC
Group

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1. PREFACE

1.1 GENERAL

Title	Contact Hypersensitivity to FAT 36'141/C in Albino Guinea Pigs. Maximization-Test.
Sponsor	Ciba Specialty Chemicals Inc. TF 2.5 Dr. M. Studer K-424.2.06 Postfach CH-4002 Basel / Switzerland
Study Monitor	Mr. E. Rüdin RCC Registration & Consulting Company Ltd. CH-4452 Itingen / Switzerland
Testing Facility	RCC, Research & Consulting Company Ltd. Zelgliweg 1, CH-4452 Itingen / Switzerland
RCC Project Number	664694
Test Article	FAT 36'141/C
Test System	Albino Guinea Pig

1.2 PROJECT STAFF

Study Director	G. Arcelin
Technical Coordinator	R. Sacher

1.3 SCHEDULE

Delivery of the Animals	29-JUL-1997 (pretest) 05-AUG-1997 (main study)
Pretest Start	04-AUG-1997
Acclimatization	11-AUG-1997 to 17-AUG-1997
Treatment / Observation	18-AUG-1997 to 15-SEP-1997
Termination	15-SEP-1997
Reported	06-NOV-1997

1.4 ARCHIVING

Research & Consulting Company Ltd., CH-4452 Itingen will archive the following data for at least 10 years: protocol, report, raw data and test article reference sample. No data will be discarded without the Sponsor's consent.

1.5 PROJECT STAFF SIGNATURES

Study Director: G. Arcelin

Date: 06-NOV-1987

Management: for T.R. Allen

Date: 06-Nov-87

1.6 QUALITY ASSURANCE STATEMENT

RCC, Research & Consulting Company Ltd., CH-4452 Itingen / Switzerland

PROJECT NUMBER	:	664694
TEST ARTICLE	:	FAT 36'141/C
STUDY DIRECTOR	:	G. Arcelin
TITLE	:	Contact Hypersensitivity to FAT 36'141/C in Albino Guinea Pigs. Maximization-Test.

Study procedures were periodically inspected and this report was audited by the RCC Quality Assurance Unit. The dates are given below.

Dates of QAU Inspections / Audits	Dates of Reports to the Study Director and to Management
24-JUL-1997	24-JUL-1997
11-AUG-1997	11-AUG-1997
10-SEP-1997	10-SEP-1997
31-OCT-1997	31-OCT-1997

Manager, Quality Assurance Unit: (for) Dr. G. Menne

M. Dörig

Date: 6 - Nov - 1997

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE / GLP GUIDELINES

PROJECT NUMBER	:	664694
TEST ARTICLE	:	FAT 36'141/C
STUDY DIRECTOR	:	G. Arcelin
TITLE	:	Contact Hypersensitivity to FAT 36'141/C in Albino Guinea Pigs. Maximization-Test.

The study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986.

OECD Principles of Good Laboratory Practice, Environment Monograph Number 45. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1992.

There were no circumstances that may have affected the quality or integrity of the data.

Study Director: G. Arcelin

Date: 06-NOV-1997

1.8 TEST GUIDELINES

The study procedures described in this report are based on the following guidelines:

Directive 96/54/EEC, B.6. "Skin Sensitization", July 30, 1996.

OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

1.9 REFERENCES

Magnusson B.; Kligman A.M., 1969.
The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. J. Invest. Dermatol. 52: 268-276.

1.10 CLASSIFICATION GUIDELINES

EEC Commission Directive 96/54/EEC, July 30, 1996 adapting to technical progress for the 22nd time Council Directive 67/548/EEC on approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

1.11 ACCREDITATION

The testing laboratory "RCC, Research & Consulting Company Ltd." is accredited according to EN 45001 under accreditation number STS 085 by the Swiss Accreditation Service.

2. SUMMARY

In order to assess the cutaneous allergenic potential of FAT 36'141/C, the Maximization-Test in accordance with OECD Guideline No. 406 and the Directive 96/54/EEC, B.6 was carried out in 15 (10 test and 5 control) female Albino guinea pigs.

The intradermal induction of sensitization was carried out with a 5 % dilution of the test article in bi-distilled water and in an emulsion with Freund's Complete Adjuvant (FCA) / physiological saline. The epidermal induction of sensitization was conducted under occlusion with the test article at 50 % in bi-distilled water. Approximately 22 hours prior to the epidermal induction the test sites of the animals were pretreated with a 10 % SLS solution in paraffinum perliquidum. Two weeks after the epidermal induction application the challenge was completed by epidermal application of the test article at 50 % in bi-distilled water under occlusive dressing. The animals of the control group were induced with bi-distilled water and FCA/physiological saline, pretreated with 10 % SLS and challenged similarly to those of the test group. Cutaneous reactions, i.e. erythema and eschar, as well as oedema formation were evaluated at 24 and 48 hours after removal of the dressing.

ERYTHEMATOUS REACTIONS AFTER THE CHALLENGE PROCEDURE

	after 24 hours	after 48 hours
	positive / total % positive of total	positive / total % positive of total
CONTROL GROUP		
FAT 36'141/C (left flank)	0 / 5 0	0 / 5 0
Bi-distilled water only (right flank)	0 / 5 0	0 / 5 0
TEST GROUP		
FAT 36'141/C (left flank)	10 / 10 100	10 / 10 100
Bi-distilled water only (right flank)	0 / 10 0	0 / 10 0

No deaths occurred.

3. CONCLUSION

In this study none of the animals of the test group were observed with positive skin reactions after treatment with a non-irritant test article concentration of 50 % in bi-distilled water. Due to a red discoloration produced by the test article a possible erythema reaction could not be determined. No skin reactions were observed in the control group.

A response of at least 30 % positive animals is considered positive "R43": may cause sensitization by skin contact according to the "Commission Directive 96/54/EEC, July 30, 1996 adapting to technical progress for the 22nd time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances".

Therefore, the test article FAT 36'141/C applied at a concentration of 50 % in bi-distilled water is considered to be a sensitizer when used under the described test conditions.

4. OBJECTIVE

4.1 PURPOSE AND RATIONALE

The purpose of this skin sensitization study was to assess the allergenic potential of FAT 36'141/C when administered to the skin of Albino guinea pigs.

This study should provide a rational basis for risk assessment of the sensitizing potential of the test article in man.

5. MATERIALS AND METHODS
Experimental Design

5.1 TEST SYSTEM

Test system	Albino Dunkin Hartley Guinea Pig, HsdPoc: DH, SPF
Rationale	Recognized by the international guidelines as a recommended test system (e.g. OECD, EEC).
Source	Harlan Nederland B.V. Postbus 167 NL-3700 AD Zeist / The Netherlands Woundenbergseweg 55 NL-3707 HW Zeist / The Netherlands
Number of animals for main study / pretest	15 females / 3 females, nulliparous and non-pregnant
Age at delivery	5 - 7 weeks
Age at beginning of pre-test/acclimatization period	6 - 8 weeks
Body weight at pretest start	Pretest groups: 424 - 437 g
Body weight at beginning of acclimatization period	Control and test group 410 - 509 g
Identification	By unique cage number and corresponding ear tags.
Randomization	Randomly selected at time of delivery.
Acclimatization	One week for the control and test group under test conditions after health examination. One week for the animals of the pretest. Only animals without any visible signs of illness were used.

The animals were distributed as follows:

	NUMBER OF ANIMALS PER GROUP	ANIMAL NUMBERS PER GROUP
1 Control Group	5	571 - 575
2 Test Group	10	576 - 585
3 Intradermal Pretest	1	586
4 Epidermal Pretest	2	587 - 588

The sensitivity and reliability of the experimental technique employed was assessed by use of a substance which is known to have skin sensitization properties in the guinea pig strain. The positive control was performed with ALPHA-HEXYLCINNAMALDEHYDE (RCC project 901596) from 06-JAN-1997 to 13-FEB-1997, see Appendix C.

5.2 HUSBANDRY

Room no.	E 22 / RCC
Conditions	**Standard Laboratory Conditions** Air-conditioned with 10-15 air changes per hour and continuously monitored environment with a target range for room temperature of 22 ± 3 °C and for relative humidity between 40-70 % (values above 70 % during cleaning process possible). The animals were provided with a 12-hour light, 12-hour dark cycle. Music was played during the light period.
Accommodation	Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz).
Diet	Pelleted standard Nafag Ecosan 845 25W4, batch nos. 37/97 and 58/97 guinea pig breeding / maintenance diet ("Nafag", Nähr- und Futtermittel AG, CH-9202 Gossau), *ad libitum*. Results of analyses for contaminants are archived at RCC.
Water	Community tap water from Itingen, *ad libitum*. Once weekly additional supply of ascorbic acid (approx. 1 g/l) via the drinking water was provided. Results of bacteriological, chemical and contaminant analyses are archived at RCC.

5.3 TEST ARTICLE (ACCORDING TO INFORMATION PROVIDED BY THE SPONSOR)

Identification	FAT 36'141/C
Description	Red powder
EN-number	007581.A7
Purity	42.3 %
Formulation or composition	Confidential information; available in Sponsor's file.
Stability of test article	Stable under storage conditions; expiration date: June 2002
Stability of test article dilution	Stable in bi-distilled water, FCA and saline for 24 hours.
Storage conditions	In the original container at room temperature (approx. 20 °C), away from direct sunlight.
Safety precautions	Gloves, goggles and face mask were obligatory to ensure the health and safety of the personnel.

5.4 TEST ARTICLE PREPARATION

The test article and vehicle were placed into a glass beaker on a tared Mettler PM 460 balance and a weight by weight dilution was prepared. Homogeneity of the test article in a suitable vehicle* was maintained during treatment using a magnetic stirrer. The preparations were made immediately prior to each dosing.

5.5 RATIONALE

The application procedure was used to detect a possible allergenic potential of the test article applied.

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* Bi-distilled water was used for the intradermal and epidermal pretests. It was also used for the intradermal and epidermal induction and the challenge in the main study. The 1:1 mixture (v/v) of Freund's Complete Adjuvant: physiological saline was used as a vehicle for the pretest and the intradermal induction in the main study.

5.6 READINGS AND SCORING

The following parameters were recorded:

Erythema (E) - 0 to 4 numerical scores
Oedema (Oe) - 0 to 4 numerical scores
Diameter (D) - mm (for the intradermal pretest)

Erythema and oedema were assessed using the following numerical grading system according to Draize (Draize J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Association of Food and Drug Officials of the United States, Austin, Texas, 1959):

ERYTHEMA AND ESCHAR FORMATION:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

OEDEMA FORMATION:	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond the area of exposure)	4

6. STUDY CONDUCT - TREATMENT PROCEDURE

6.1 PRETEST / PERFORMED DURING THE ACCLIMATIZATION PERIOD

The objective of this investigation was to identify a maximally tolerated concentration of the test article suitable for the induction phase of the main study. In addition, a suitable non-irritant concentration of the test article, by the topical route of administration, was identified for the challenge application. The concentrations tested were for the epidermal application the most qualified to assure an optimum technical application procedure and for the intradermal injection the selected concentrations were tested up to 5 % (The Guinea Pig Maximization-Test, page 270. Magnusson B.; Kligman A.M., 1969).

The procedure employed for these investigations was as follows:

INTRADERMAL INJECTIONS:

Four intradermal injections (0.1 ml/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant/physiological saline were made into the shaved neck of one guinea pig. One week later intradermal injections (0.1 ml/site) were made into the clipped flank of the same guinea pig at concentrations of 5, 3 and 1 % of the test article in bi-distilled water.
The resulting dermal reactions were assessed 24 hours later. For intradermal induction application in the main study a 5 % test article concentration was selected.

EPIDERMAL APPLICATIONS:

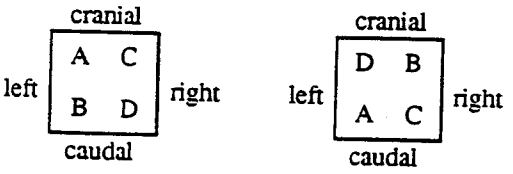
Four intradermal injections (0.1 ml/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant/physiological saline were made into the shaved neck of two guinea pigs. One week later both flanks of each of the guinea pigs were clipped and shaved just prior to the application. Thereafter 4 patches of filter paper (2 x 2 cm) were saturated with the test article at A = 50 % (this concentration was found to be the most qualified to assure an optimum technical application procedure), B = 25 %, C = 15 % and D = 10 % in bi-distilled water and applied to the clipped and shaved flanks. The volume of test article applied at 50 % was approximately 0.2 g. The volume of the remaining test article concentrations was 0.2 ml. The patches were covered by a strip of aluminum foil and firmly secured by elastic plaster wrapped around the trunk and covered with impervious adhesive tape. This procedure ensured the intensive contact of the test article. The dressings were removed after an exposure period of 24 hours.

Approximately 21 hours after removal of the dressing the application site was depilated with an approved depilatory cream (VEET Cream, Reckitt & Colman AG, CH-4123 Allschwil) to clean the application site from staining produced by the test article, so that possible erythema reactions were clearly visible at that time.

The depilatory cream was placed on the patch sites and surrounding areas, and left on for 3-5 minutes. It was then thoroughly washed off with a stream of warm, running water. The animals were then dried with a disposable towel, and returned to their cages.

The reaction sites were assessed 24 and 48 hours after removal of the bandage for erythema and oedema on a numerical basis according to Draize described above.

The position of the epidermal applications is shown below:



Animal no. 587 Animal no. 588

The allocation of the different test dilutions to the sites (A, B, C, D) on the animals was alternated in order to minimize site-to-site variation in responsiveness.

The concentration selected for the induction period and challenge procedure was 50 %.

6.2 MAIN STUDY

6.2.1 INDUCTION

6.2.1.1 INTRADERMAL INJECTIONS / PERFORMED ON TEST DAY 1

An area of dorsal skin from the scapular region (approximately 6 x 8 cm) was clipped free of hair. Three pairs of intradermal injections (0.1 ml/site) were made at the border of a 4 x 6 cm area in the clipped region as follows:

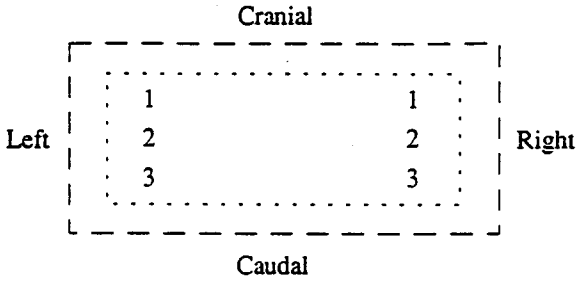
Test Group:

1) 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.
2) The test article, diluted to 5 % with bi-distilled water.
3) The test article diluted to 5 % by emulsion in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

Control Group:

1) 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.
2) Bi-distilled water.
3) 1:1 (w/w) mixture of bi-distilled water in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

The positions of the intradermal injections are shown below:



— — — — — — — clipped area
. . . . . area in which injections were made

6.2.1.2 EPIDERMAL APPLICATIONS / PERFORMED ON TEST DAY 8

On test day 7 and approximately 21.5 hours prior to the epidermal application the scapular area (approximately 6 x 8 cm) of the animals of the control and test group was clipped, shaved free of hair and the test area was pretreated with a 10 % dilution of Sodium-Lauryl-Sulfate (SLS) in paraffinum perliquidum as no primary irritation had been observed in the pretest. The SLS was massaged into the skin with a glass rod without bandaging. This 10 % concentration of SLS enhances sensitization by provoking a mild inflammatory reaction (Magnusson and Kligman 1970).

One week after the injections, the scapular area (approximately 6 x 8 cm) was again clipped and shaved free of hair. A 2 x 4 cm patch of filter paper was saturated with the test article (50 % in bi-distilled water) and placed over the injection sites of the test animals. The volume of test article applied was approximately 0.3 g. The patch was covered with aluminum foil and firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The dressings were left in place for 48 hours. The epidermal application procedure described ensured intensive contact of the test article.

The guinea pigs of the control group were treated as described above with bi-distilled water only.

Reaction sites were assessed for erythema and oedema 24 and 48 hours after removal of the dressing, using the numerical grading system according to Draize.

6.2.2 CHALLENGE / PERFORMED ON TEST DAY 22

The test and control guinea pigs were challenged two weeks after the epidermal induction application. The test and control guinea pigs were treated in the same way.

Hair was clipped and shaved from a 5 x 5 cm area on the left and right flank of each guinea pig just prior to the application. Two patches (2 x 2 cm) of filter paper were saturated with the highest non-irritating concentration of 50 % (left flank) and the vehicle only (bi-distilled water applied to the right flank) using the same method as for the epidermal application. The volume of test article applied was approximately 0.2 g. The dressings were left in place for 24 hours.

Approximately 21 hours after removal of the dressing the test sites treated with the test article were depilated as described in the epidermal pretest.

Approximately 24 and 48 hours after the removal of the dressing the application sites were assessed for erythema and oedema using the numerical scoring system according to Draize.

Erythema and oedema reactions are described in the tables under Appendix A.

6.3 INTERPRETATION

The results obtained from test animals following the challenge applications were compared with the results seen in control animals.

An allergic reaction was defined by visible reddening of the challenge site.

If the dermal reactions of test animals following the challenge were more marked and/or persistent than those of the control animals, the animals were considered to show evidence of contact hypersensitivity.

If the dermal reactions of test animals following the challenge were not clearly different from the reactions seen in the control group animals, the results for the test animals were considered "inconclusive".

The test animals were considered to show no evidence of contact hypersensitivity if the dermal reactions to the challenge application were identical to or less marked and/or persistent than the reactions observed in the control animals.

By "maximizing" the exposure and enhancing allergenicity, some problems could arise, particularly in relation to specificity, especially the potential for false-positive reactions. An inflammatory response at challenge may not necessarily be due to allergenicity, but instead may be a false-positive irritant response caused by an inducing hyperirritability.

6.4 READING OF CHALLENGE REACTIONS

The challenge site was evaluated 24 and 48 hours after the removal of the patch. The readings were made under artificial fluorescent light (daylight spectrum).

Redness constitutes the minimum criterion of an allergic reaction. Strongly sensitized animals display a vivid redness, associated with indurated swelling. The reactions were scored on the basis of the Draize score described under "Readings and Scoring".

6.5 RATING OF ALLERGENICITY

According to Magnusson and Kligman:

Based upon the percentage of animals sensitized (24- and 48-hour reading), the test article was assigned to one of the following five grades of allergenic potency according to Magnusson and Kligman, ranging from weak to extreme:

Sensitization Rate (%)	Grade	Classification
0 - 8	1	weak
9 - 28	2	mild
29 - 64	3	moderate
65 - 80	4	strong
81 - 100	5	extreme

According to the "Commission Directive 96/54/EEC, July 30, 1996 adapting to technical progress for the 22nd time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances".

A response of at least 30 % positive animals is considered positive "R43": may cause sensitization by skin contact.

6.6 OBSERVATIONS

In addition to the sensitizing reactions the following observations and data were recorded during the test and observation period:

Viability / Mortality	Daily from delivery of the animals to the termination of test.
Clinical signs (local / systemic)	Daily from delivery of the animals to the termination of test.
Skin reactions	At the times specified during the induction and challenge periods.
Body weights	At pretest start, one week prior to day 1 (main study), day 1 and termination of the test.

Records were maintained of all additional and standard observations.

6.7 PATHOLOGY

NECROPSY

No necropsies were performed in the animals of the control and test group sacrificed at termination of the observation period and in the animals of the intradermal and epidermal pretest sacrificed on test day 1 of the main study.

The animals were sacrificed by intraperitoneal injection of NARCOREN (Rhône Merieux GmbH, D-88471 Laupheim) at a dose of at least 5.1 ml/kg body weight (equivalent to 810 mg sodium pentobarbitone/kg body weight) and discarded.

6.8 STATISTICAL ANALYSIS

No statistical analysis was performed. Only mean values with standard deviations were described in the body weight tables.

6.9 DATA COMPILATION

The following data were compiled into the RCC computer system during recording:

skin reactions,
viability / mortality,
clinical signs (local / systemic).

The following data were recorded on-line:

body weights.

7. RESULTS
Main Study

7.1 SKIN EFFECTS AFTER INTRADERMAL INDUCTION - PERFORMED ON TEST DAY 1

A normal development of the expected local symptoms was observed in the animals of the control and test group after the intradermal injections.

7.2 SKIN EFFECTS AFTER EPIDERMAL INDUCTION - PERFORMED ON TEST DAY 8

CONTROL AND TEST GROUP

No erythematous or oedematous reaction was observed in the animals either when treated with bi-distilled water only or when treated with the test article at 50 % in bi-distilled water.

As the test article stained the skin red, it was not possible to determine whether erythema was present or not. However no oedema was observed.

All animals of the control and test group were pretreated with 10 % SLS in paraffinum perliquidum.

See pp. 27-28

7.3 SKIN EFFECTS AFTER THE CHALLENGE - PERFORMED ON TEST DAY 22

CONTROL GROUP

No positive reactions were observed in the animals either when treated with bi-distilled water only or when treated with the test article at 50 % in bi-distilled water.

Red discoloration was noted directly after removal of the patch. To remove discoloration all animals were depilated approximately 3 hours prior to challenge reading.

TEST GROUP

Positive reactions were observed in all animals (nos. 576-585) at the 24- and 48-hour reading when treated with the test article at 50 % in bi-distilled water. No positive reactions were observed in the animals when treated with bi-distilled water only.

Red discoloration was noted directly after removal of the patch. To remove discoloration all animals were depilated approximately 3 hours prior to challenge reading.

See pp. 29-32

7.4 VIABILITY / MORTALITY / MACROSCOPIC FINDINGS

As there were no deaths during the course of the treatment period no necropsies were performed.

7.5 CLINICAL SIGNS, SYSTEMIC

No symptoms of systemic toxicity were observed in the animals.

7.6 BODY WEIGHTS

The body weight of the animals was within the range of physiological variability known for guinea pigs of this strain and age.

See pp. 34-37

APPENDIX A

PRETEST

MAIN STUDY - INDUCTION - EPIDERMAL REACTIONS
- CHALLENGE - EPIDERMAL REACTIONS

PRETEST

The following reactions were observed in the pretest:

INTRADERMAL INJECTION / performed during the acclimatization period

Vehicle: Bi-distilled water

Animal No.	Sex	Concentration %	REACTION READINGS AFTER 24 HOURS		
			Erythema	Oedema	Diameter (mm)
586	F	5	1	1	7 x 7
		3	1	1	5 x 5
		1	1	1	4 x 5

According to Magnusson - Kligman and to the findings observed, the concentration selected for the main study was 5 %.

PRETEST (CONTINUED)

EPIDERMAL PRETEST / performed during the acclimatization period

Vehicle: Bi-distilled water

Animal No.	Sex	Concentration %	REACTION READINGS AFTER REMOVAL OF BANDAGE			
			After 24 hours		After 48 hours	
			E	Oe	E	Oe
587	F	50	0	0	0	0
		25	0	0	0	0
		15	0	0	0	0
		10	0	0	0	0
588	F	10	0	0	0	0
		50	0	0	0	0
		25	0	0	0	0
		15	0	0	0	0

E = Erythema
Oe = Oedema

Approximately 21 hours after removal of the dressing the test sites were depilated.

According to Magnusson - Kligman and to the findings observed, the concentration selected for the induction period and challenge procedure was 50 %.

MAIN STUDY - INDUCTION

TABLE 1: CONTROL GROUP

SKIN RESPONSE AFTER THE EPIDERMAL APPLICATION OF THE VEHICLE (BI-DISTILLED WATER) DURING INDUCTION PERIOD (SCAPULAR AREA)

Animal No.	Sex	Erythema / Oedema Readings after removal of bandage			
		24 hours		48 hours	
		E	Oe	E	Oe
571	Female	0	0	0	0
572	Female	0	0	0	0
573	Female	0	0	0	0
574	Female	0	0	0	0
575	Female	0	0	0	0

E = Erythema
Oe = Oedema

The animals of the control group were treated with a 10 % Sodium-Lauryl-Sulfate solution approximately 21.5 hours prior to the epidermal induction application.

MAIN STUDY - INDUCTION (CONTINUED)

TABLE 2: TEST GROUP

SKIN RESPONSE AFTER THE EPIDERMAL APPLICATION OF FAT 36'141/C
(50 % IN BI-DISTILLED WATER) DURING INDUCTION PERIOD (SCAPULAR AREA)

Animal No.	Sex	Erythema / Oedema Readings after removal of bandage			
		24 hours		48 hours	
		E*	Oe	E*	Oe
576	Female	-	0	-	0
577	Female	-	0	-	0
578	Female	-	0	-	0
579	Female	-	0	-	0
580	Female	-	0	-	0
581	Female	-	0	-	0
582	Female	-	0	-	0
583	Female	-	0	-	0
584	Female	-	0	-	0
585	Female	-	0	-	0

E = Erythema
Oe = Oedema

* Due to a red discoloration produced by the test article a possible erythema reaction could not be determined.

The animals of the test group were treated with a 10 % Sodium-Lauryl-Sulfate solution approximately 21.5 hours prior to the epidermal induction application.

MAIN STUDY - CHALLENGE

TABLE 3: CONTROL GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF BI-DISTILLED WATER (RIGHT FLANK)

Animal No.	Sex	Erythema / Oedema Readings after removal of bandage			
		24 hours		48 hours	
		E	Oe	E	Oe
571	Female	0	0	0	0
572	Female	0	0	0	0
573	Female	0	0	0	0
574	Female	0	0	0	0
575	Female	0	0	0	0

E = Erythema
Oe = Oedema

MAIN STUDY - CHALLENGE (CONTINUED)

TABLE 4: CONTROL GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF FAT 36'141/C, 50 % IN BI-DISTILLED WATER (LEFT FLANK)

Animal No.	Sex	Erythema / Oedema Readings after removal of bandage			
		24 hours		48 hours	
		E	Oe	E	Oe
571	Female	0	0	0	0
572	Female	0	0	0	0
573	Female	0	0	0	0
574	Female	0	0	0	0
575	Female	0	0	0	0

E = Erythema
Oe = Oedema

Approximately 21 hours after removal of the dressing the test sites were depilated.

MAIN STUDY - CHALLENGE (CONTINUED)

TABLE 5: TEST GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF BI-DISTILLED WATER (RIGHT FLANK)

Animal No.	Sex	Erythema / Oedema Readings after removal of bandage			
		24 hours		48 hours	
		E	Oe	E	Oe
576	Female	0	0	0	0
577	Female	0	0	0	0
578	Female	0	0	0	0
579	Female	0	0	0	0
580	Female	0	0	0	0
581	Female	0	0	0	0
582	Female	0	0	0	0
583	Female	0	0	0	0
584	Female	0	0	0	0
585	Female	0	0	0	0

E = Erythema
Oe = Oedema

MAIN STUDY - CHALLENGE (CONTINUED)

TABLE 6: TEST GROUP

SKIN RESPONSE AFTER THE CHALLENGE APPLICATION OF FAT 36'141/C, 50 % IN BI-DISTILLED WATER (LEFT FLANK)

Animal No.	Sex	Erythema / Oedema Readings after removal of bandage			
		24 hours		48 hours	
		E	Oe	E	Oe
576	Female	1	0	1	0
577	Female	2	0	2	0
578	Female	2	0	2	0
579	Female	1	0	1	0
580	Female	2	0	2	0
581	Female	1	0	1	0
582	Female	1	0	1	0
583	Female	2	0	2	0
584	Female	2	0	2	0
585	Female	1	0	1	0

E = Erythema
Oe = Oedema

Approximately 21 hours after removal of the dressing the test sites were depilated.

APPENDIX B

BODY WEIGHTS

- SUMMARY

- INDIVIDUAL

RCC PROJECT 664694
FAT 36'141/C

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

PRETEST		GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP	GROUP 3 INTRADERMAL PRETEST
DAY 1	MEAN	---	---	424
WEEK 1	ST.DEV.	---	---	---
	MINIMUM	---	---	424
	MAXIMUM	---	---	424
	N	0	0	1

	GROUP 4 EPIDERMAL PRETEST
MEAN	431
ST.DEV.	7.2
MINIMUM	426
MAXIMUM	437
N	2

RCC PROJECT 664694
FAT 36'141/C

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

ACCLIMATIZATION		GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP	GROUP 3 INTRADERMAL PRETEST
DAY 1	MEAN	432	450	474
WEEK 1	ST.DEV.	19.4	31.6	---
	MINIMUM	414	410	474
	MAXIMUM	465	509	474
	N	5	10	1

	GROUP 4 EPIDERMAL PRETEST
MEAN	452
ST.DEV.	22.1
MINIMUM	436
MAXIMUM	468
N	2

RCC PROJECT 664694
FAT 36'141/C

BODY WEIGHTS (GRAM) SUMMARY
FEMALES

TREATMENT		GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP	GROUP 3 INTRADERMAL PRETEST
DAY 1 WEEK 1	MEAN	484	493	527
	ST.DEV.	28.1	25.1	---
	MINIMUM	464	454	527
	MAXIMUM	533	526	527
	N	5	10	1

	GROUP 4 EPIDERMAL PRETEST
MEAN	431
ST.DEV.	70.8
MINIMUM	381
MAXIMUM	481
N	2

		GROUP 1 CONTROL GROUP	GROUP 2 TEST GROUP	GROUP 3 INTRADERMAL PRETEST
DAY 29 WEEK 5	MEAN	562	563	---
	ST.DEV.	35.3	25.1	---
	MINIMUM	526	519	---
	MAXIMUM	619	598	---
	N	5	10	0

	GROUP 4 EPIDERMAL PRETEST
MEAN	---
ST.DEV.	---
MINIMUM	---
MAXIMUM	---
N	0

RCC PROJECT 664694
FAT 36'141/C

BODY WEIGHTS (GRAM)
FEMALES

	PRETEST	ACCLIMATIZATION	TREATMENT	
DAYS	1	1	1	29
WEEKS	1	1	1	5
ANIMAL				
GROUP 1 (CONTROL GROUP)				
571	---	433	472	554
572	---	465	533	619
573	---	424	470	544
574	---	424	464	526
575	---	414	482	565
GROUP 2 (TEST GROUP)				
576	---	418	454	555
577	---	433	526	567
578	---	509	524	598
579	---	428	474	519
580	---	474	505	588
581	---	410	456	543
582	---	435	498	539
583	---	447	507	564
584	---	485	493	591
585	---	458	497	567
GROUP 3 (INTRADERMAL PRETEST)				
586	424	474	527	---
GROUP 4 (EPIDERMAL PRETEST)				
587	437	468	481	---
588	426	436	381	---

APPENDIX C

RESULTS OF POSITIVE CONTROL

RCC PROJECT 664694
FAT 36'141/C

RCC PROJECT 901596

CONTACT HYPERSENSITIVITY TO

ALPHA-HEXYLCINNAMALDEHYDE

IN ALBINO GUINEA PIGS
MAXIMIZATION-TEST

POSITIVE CONTROL

SUMMARY

For validation of sensitivity of the Maximization Test of B. Magnusson and A.M. Kligman (1969) as well as the sensitivity of the test system used, a known sensitizer was selected as a positive control. This was performed in accordance with the recommendation of the OECD for testing of chemicals number 406 "Skin Sensitization Test", adopted by the council on July 17, 1992.

The raw data from this project are kept in a separate file at RCC. The test described was performed under GLP-conditions with a final QA-check.

Test article description:

Identification	ALPHA-HEXYLCINNAMALDEHYDE
Description	Clear liquid
Lot number	10021HF
Purity	85 %
Formulation	Unknown
Stability of test article	Unknown
Stability of test article in vehicle	Unknown in polyethylene glycol (PEG 400) and in a 1:1 (v/v) mixture of FCA: physiological saline
Storage conditions	In the original container, at room temperature No specific conditions

Test system:

Albino Dunkin Hartley Guinea Pig, CRL:(HA)BR SPF
delivered by
Charles River Deutschland GmbH
Stolzenseeweg 32-36
D-88353 Kisslegg / Germany

Five males were used as control group and ten males were used as test group.

The intradermal induction was performed with a dilution at 5 % in polyethylene glycol (PEG 400).
For the epidermal induction period a 25 % dilution was selected.

RESULTS

The non-irritating test article concentration used for challenge application was 25 % in polyethylene glycol (PEG 400).

According to the procedures used in this experiment (performed from 06-JAN-1997 to 13-FEB-1997) positive results were observed in the treated animals after the epidermal challenge application.

ERYTHEMATOUS REACTIONS AFTER THE CHALLENGE PROCEDURE

	after 24 hours	after 48 hours
	positive / total % positive of total	positive / total % positive of total
CONTROL GROUP		
ALPHA-HEXYLCINNAMALDEHYDE (left flank)	0 / 5 0	0 / 5 0
Polyethylene glycol (PEG 400) only (right flank)	0 / 5 0	0 / 5 0
TEST GROUP		
ALPHA-HEXYLCINNAMALDEHYDE (left flank)	7 / 10 70	7 / 10 70
Polyethylene glycol (PEG 400) only (right flank)	0 / 10 0	0 / 10 0

No toxic symptoms were evident in the guinea pigs of the control or test group.

No deaths occurred.

CONCLUSION

In this study 70 % of the animals of the test group were observed with positive skin reactions after treatment with a non-irritant test article concentration of 25 % in polyethylene glycol (PEG 400). No skin reactions were observed in the control group.

A response of at least 30 % positive animals is considered positive "R43": may cause sensitization by skin contact according to the "Commission Directive 96/54/EEC, July 30, 1996 adapting to technical progress for the 22nd time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances".
Therefore, the test article ALPHA-HEXYLCINNAMALDEHYDE applied at a concentration of 25 % in polyethylene glycol (PEG 400) is considered to be a sensitizer when used under the described test conditions.

According to the rating of allergenicity by Magnusson and Kligman the test article is a strong sensitizer.

MAIN STUDY - CHALLENGE

TEST GROUP

SKIN RESPONSE AFTER THE CHALLENGE PROCEDURE OF ALPHA-HEXYLCINNAMALDEHYDE, 25 % IN POLYETHYLENE GLYCOL (PEG 400) (LEFT FLANK)

Animal No.	Sex	Erythema / Oedema Readings after removal of bandage			
		24 hours		48 hours	
		E	Oe	E	Oe
381	Male	1	0	1	0
382	Male	2	0	2	0
383	Male	0	0	0	0
384	Male	1	0	1	0
385	Male	1	0	1	0
386	Male	1	0	1	0
387	Male	2	0	2	0
388	Male	2	0	2	0
389	Male	0	0	0	0
390	Male	0	0	0	0

E = Erythema
Oe = Oedema

APPENDIX D

SUMMARY TABLE OF STUDY INFORMATION AND RESULTS

Test article identification: Name: FAT 36'141/C			SUMMARY TABLE	
Batch No.: 007581.A7				
SKIN TOLERANCE STUDIES / IMMUNOSTIMULATION (sensitization potential by intradermal and epidermal administration) **Maximization Test (MT)**			RCC Project No.: 664694 Report Date: 06-NOV-1997	
Species/Strain:	Albino Dunkin Hartley Guinea Pig, HsdPoc: DH, SPF		Number of exp. animals: 15	
Procedure	Administration route/site		Day	Vehicle
First induction	intradermal/scapular		1	Test: 1. FCA:phys. saline 1:1 2. Bi-distilled water 3. FCA:phys. saline 1:1 Control: 1. FCA:phys. saline 1:1 2. Bi-distilled water 3. FCA:phys. saline 1:1 / bi-distilled water 50/50
Second induction	Epidermal occl./scapular		8	} Bi-distilled water
Challenge	Epidermal occl./left flank		22	
Study group	Control group		Test group	
	Application	No. of appl. and dose	Conc. of test art. in %	No. of appl. and dose
Intradermal induction 1.	FCA:phys. saline 1:1	2x100 µl/i.d.	FCA:phys. saline 1:1	2x100 µl/i.d.
2.	Bi-distilled water	2x100 µl/i.d.	5 %	2x100 µl/i.d.
3.	FCA:phys. saline 1:1/bi-distilled water 50/50	2x100 µl/i.d.	5 %	2x100 µl/i.d.
Epidermal occl. patch induction	Bi-distilled water	Saturated patch/8 cm^2	50 %	Saturated patch/8 cm^2
Challenge A	50 %	} Saturated patch/4 cm^2	50 %	} Saturated patch/4 cm^2
B	Bi-distilled water		Bi-distilled water	
Number of animals and Sex	5 females		10 females	
Animals with erythematous reactions / out of total				
Challenge A (24-hour reading)	0 / 5		10 / 10	
B	0 / 5		0 / 10	
Challenge A (48-hour reading)	0 / 5		10 / 10	
B	0 / 5		0 / 10	
Summary of salient findings: The test article tested under the described conditions is considered to be a sensitizer.				
Study conducted by the applicant: yes ☐ no ☒				
Study in compliance with GLP: yes ☒ no ☐			QAU inspected: yes ☒ no ☐	

A = left flank B = right flank

APPENDIX E

CERTIFICATION

- ACCREDITATION / EUROPEAN STANDARD EN 45001
- GLP – CERTIFICATION

RCC PROJECT 664694
FAT 36'141/C

S SCHWEIZERISCHER PRÜFSTELLENDIENST
T SERVICE SUISSE D'ESSAI
S SERVICIO DI PROVA IN SVIZZERA
SWISS TESTING SERVICE

ACCREDITATION

EUROPEAN STANDARD EN 45001

RCC Research & Consulting Company Ltd.
Zelgliweg 1
CH-4452 Itingen/BL

This study is performed by the Testing Laboratory for the toxicological investigation of Pharmaceuticals and Medical Devices, Agrochemicals, Industrial Chemicals, Food- and Feed-Additives in accordance with

SN EN 45001

under accreditation number

STS 085

The accredited scope of testing is defined in the "STS Directory of the Swiss Accreditation Service".

To comply with this European Standard RCC is obliged to make the following statements:

- The test results relate only to the items tested.
- Information on the error of measurement (confidence interval, where relevant) can be requested.
- This report shall not be reproduced, except in full, without the written approval of the testing laboratory.

RCC PROJECT 664694
FAT 36'141/C

EIDGENÖSSISCHES DEPARTEMENT DES INNERN
DÉPARTEMENT FÉDÉRAL DE L'INTÉRIEUR
DIPARTIMENTO FEDERALE DELL'INTERNO

GLP Compliance Statement

It is hereby certified that

on February 12-16, 1996
February 19-23, 1996
June 14, 1996

the testing facilities of RCC Holding Company Ltd
4414 Füllinsdorf
Switzerland

were inspected by the Federal Office of Public Health, the Federal Office of Environment, Forests and Landscape and the Intercantonal Office for the Control of Medicaments with respect to the compliance with the Swiss GLP Principles. The inspection was performed in agreement with the OECD Guidelines for National GLP Inspections and Audits and comprised the following testing facilities:

- RCC Research and Consulting Company Ltd, Itingen
- RCC Umweltchemie AG, Itingen
- RCC Pharmanalytics Ltd, Itingen
- BRL Biological Research Laboratories Ltd/Microbiology, Füllinsdorf

It was found that the aforementioned testing facilities were operating in compliance with the Swiss Principles of Good Laboratory Practice (Good Laboratory Practice [GLP] in Switzerland, Procedures and Principles, March 1986) at the time they were inspected.

FEDERAL DEPARTMENT OF THE INTERIOR

Bern, July 9, 1996

Ruth Dreifuss
Federal Councillor